

*File E1* →  
--89. A method of expanding embryonic stem cells, said method comprising expanding embryonic stem cells in serum-free culture.

90. The method of claim 89, wherein said expanding occurs in the absence of leukemia inhibitory factor.

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91. A composition comprising embryonic stem cells in a serum-free eukaryotic cell culture medium, wherein said serum-free medium is capable of supporting the growth of embryonic stem cells in serum-free culture.

92. The composition according to claim 91, wherein said medium comprises a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements.

93. The composition according to claim 91, wherein said serum-free medium is obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one

or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements.

94. The composition according to claim 92 or 93, wherein said composition is capable of being stored indefinitely at less than or equal to about -135°C.

95. The composition according to claim 94, wherein said embryonic stem cells are obtained from an animal selected from the group consisting of human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, and amphibian.

96. The composition according to claim 95, wherein said embryonic stem cells are obtained from an animal selected from the group consisting of mouse, cow, goat, and sheep.

97. The composition according to claim 96, wherein said embryonic stem cells are obtained from mouse.

98. A product of manufacture comprising a container means containing embryonic stem cells and a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more

transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements, wherein a basal cell culture medium supplemented with said supplement is capable of supporting the growth of embryonic stem cells in serum-free culture.

99. A product of manufacture comprising a container means containing embryonic stem cells in a serum-free eukaryotic cell culture medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements, wherein said supplemented medium is capable of supporting the growth of embryonic stem cells in serum-free culture.

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100. A product of manufacture comprising a container means containing embryonic stem cells in a serum-free medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said serum-free medium is capable of supporting the growth of embryonic stem cells in serum-free culture.

101. A product of manufacture comprising one or more container means, wherein a first container means contains a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein a basal cell culture medium supplemented with said supplement is capable  
of supporting the growth of embryonic stem cells in serum-free culture,

wherein optionally a second container means contains a basal medium, and  
wherein optionally a third container means contains embryonic stem cells.

102. A product of manufacture comprising one or more container means, wherein a first container means contains a serum-free eukaryotic cell culture medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said supplemented medium is capable of supporting the growth of embryonic stem cells in serum-free culture, and

wherein ~~optionally~~ a second container means contains embryonic stem cells.

103. A product of manufacture comprising one or more container means, wherein a first container means contains a serum-free medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said serum-free medium <sup>supports</sup> ~~is capable of supporting~~ the growth of embryonic stem cells in serum-free culture, and

wherein ~~optionally~~ a second container means contains embryonic stem cells.

104. The product of manufacture according to any one of claims 98-103, wherein said product of manufacture is in a frozen state.

*Sub F1*  
105. A method of expanding embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected

from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion of said embryonic stem cells.

*106.* A method of expanding embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion of said embryonic stem cells.

*107.* The method according to claim 105 or 106, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

*File #1* 108. A method for controlling or preventing the differentiation of embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements; and

*Ar* (b) cultivating said embryonic stem cells under serum-free conditions suitable to control or prevent the differentiation of embryonic stem cells and facilitate the expansion of said embryonic stem cells in serum-free culture.

109. A method for controlling or preventing the differentiation of embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to control or prevent the differentiation of embryonic stem cells and facilitate the expansion of said embryonic stem cells in serum-free culture.

110. The method according to claim 108 or 109, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

111. The method according to claim 110, wherein said cultivating further comprises supplementing said medium with one or more factors which control or prevent the differentiation of said embryonic stem cells.

112. The method according to claim 111, wherein said factor is selected from the group consisting of leukemia inhibitory factor, steel factor, ciliary neurotrophic factor, and oncostatin M.

113. The method according to claim 112, wherein said factor is leukemia inhibitory factor.

114. The method according to claim 112, wherein said factor is steel factor.

115. The method according to claim 112, wherein said factor is ciliary neurotrophic factor.

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116. The method according to claim 112, wherein said factor is oncostatin M.

*July 11/* 117. A method of causing embryonic stem cells to differentiate into a particular type of cell in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements;

(b) cultivating said embryonic stem cells under conditions suitable to facilitate the expansion of embryonic stem cells in serum-free culture; and

(c) adding a differentiation factor or changing culturing conditions to induce differentiation of embryonic stem cells to form a different type of cell.

118. A method of causing embryonic stem cells to differentiate into a particular type of cell in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more

antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements;

- (b) cultivating said embryonic stem cells under conditions suitable to facilitate the expansion of embryonic stem cells in serum-free culture; and
- (c) adding a differentiation factor or changing culturing conditions to induce differentiation of embryonic stem cells to form a different type of cell.

119. The method according to claim 117 or 118, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

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120. The method according to claim 117 or 118, wherein said cultivating said embryonic stem cells under conditions suitable to prevent the differentiation of and facilitate the expansion of said cells further comprises supplementing said culture medium with one or more growth factors which prevent differentiation of said embryonic stem cells.

121. The method according to claim 117 or 118, wherein said cultivating said embryonic stem cells further comprises supplementing said culture medium with one or more growth factors which facilitate differentiation of said embryonic stem cells.

*File #1*  
122. A method of obtaining embryonic stem cells in serum-free culture, said method comprising

- (a) isolating embryonic stem cells from blastocysts; and

(b) cultivating said isolated embryonic stem cells in a serum-free eukaryotic cell culture medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said supplemented medium *is capable of supporting* the growth of embryonic stem cells in serum-free culture.

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123. A method of obtaining embryonic stem cells in serum-free culture, said method comprising

(a) isolating embryonic stem cells from blastocysts; and  
(b) cultivating said isolated embryonic stem cells in a serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said serum-free medium *is capable of supporting* the growth of embryonic stem cells in serum-free culture.